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Transient receptor potential channel modulators as pharmacological treatments for lower urinary tract symptoms (LUTS): myth or reality?

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Transient receptor potential (TRP) channels belong to the most intensely pursued drug targets of the last decade. These ion channels are considered promising targets for the treatment of pain, hypersensitivity disorders and lower urinary tract symptoms (LUTS). The aim of the present review is to discuss to what extent TRP channels have adhered to their promise as new pharmacological targets in the lower urinary tract (LUT) and to outline the challenges that lie ahead.

- TRP vanilloid 1 (TRPV1) agonists have proven their efficacy in the treatment of neurogenic detrusor overactivity (DO), albeit at the expense of prolonged adverse effects as pelvic 'burning' pain, sensory urgency and haematuria.
- TRPV1 antagonists have been very successful in preclinical studies to treat pain and DO. However, clinical trials with the first generation TRPV1 antagonists were terminated early due to hyperthermia, a serious, on-target, side-effect.
- TRP vanilloid 4 (TRPV4), TRP ankyrin 1 (TRPA1) and TRP melastatin 8 (TRPM8) have important sensory functions in the LUT. Antagonists of these channels have shown their potential in pre-clinical studies of LUT dysfunction and are awaiting clinical validation.

Keywords

TRP channel, afferent nerve, urothelium, detrusor overactivity, LUTS, TRPV1, TRPV4

Introduction: Transient Receptor Potential (TRP) Channels as Therapeutic Targets in Functional Lower Urinary Tract (LUT) Disorders

TRP channels constitute a large superfamily of cation selective channels. The human TRP family consists of 27 members that are divided in six subfamilies: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin) and TRPA (ankyrin) [1].

A particular characteristic of these ubiquitously expressed channels is that a wide range of physical and chemical stimuli can regulate their activation. Thus, TRP channels can act as polymodal cellular sensors that are involved in numerous sensory and homeostatic processes. Recent research has shown the involvement of TRP channels in numerous physiological processes and in the ontogeny of hereditary and acquired diseases [1]. Therefore, TRP channels represent a promising class of pharmacological targets for various pathological conditions.

TRP channels in the LUT have been increasingly studied over the last decade. Their (patho)physiological role in LUT (dys)function is slowly being deciphered and some of them are considered attractive targets for pharmacotherapy of LUT dysfunction. TRP channels that function as cellular sensors in the bladder wall can directly be targeted to modulate mechanosensation (feelings or urge or urgency), pain perception (suprapubic pain, mictalgia . . .) and/or thermosensation ('burning' sensations, bladder cooling reflex). Alternatively, these proteins can be targeted to alter the function (excitability) of sensory nerves that send afferent information from the LUT to the CNS. Growing evidence suggests that these afferent neural pathways are at least as important as efferent ortho- and parasympathetic nerves in controlling the voluntary control of micturition and the pathogenesis of functional LUT diseases [2]. The aim of this

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review is to critically appraise the current knowledge about the role of TRP channels in LUT dysfunction and their potential as targets for new drug therapies.

TRPV1

TRPV1, also known as the vanilloid receptor type 1, was identified as a heat-sensitive ion channel that can also be activated by capsaicin, the pungent compound of 'hot' chilli peppers [3]. TRPV1 is highly expressed in unmyelinated, small diameter sensory fibres (C-fibres) of cranial and spinal nerves innervating skin, muscles and visceral organs. Activation of this channel depolarises TRPV1-expressing nociceptive neurones, causing a painful, burning sensation. Moreover, these sensory neurones will release pro-inflammatory neuropeptides [calcitonin gene-related peptide (CGRP), substance P, neurokinins] inducing so-called neurogenic inflammation [4].

The channel is activated by a myriad of stimuli, including heat, extracellular acidification and binding of specific endogenous (anandamide, N-arachidonoyl) or exogenous ligands [capsaicin, resiniferatoxin (RTX)], making it a molecular integrator of multiple noxious stimuli [1].

TRPV1 in the LUT

TRPV1 is highly expressed in C-fibres innervating the urinary bladder and urethra in several mammals including rats [5], mice [6] and humans [7].

Although it has been suggested that TRPV1 is also expressed in non-neuronal cell types [8], the functional expression of TRPV1 in the urothelium is very controversial. Initial reports described TRPV1 expression and functional TRPV1 responses in isolated urothelial cells from mice [8], rats [9] and humans [10]. In contrast, an increasing number of groups have more recently reported the absence of TRPV1 expression and functional TRPV1 responses in guinea-pig [8], mouse [11–15] and human [16] urothelial cells. Moreover, a recent approach using *Trpv1*-reporter mice, suggested that TRPV1 expression was restricted to primary afferent neurones, with no detectable TRPV1 expression in urothelial cells [17].

There can be multiple reasons for these apparent contradictions: First, Yu et al. [6] have suggested that TRPV1 is present on afferent neurones that extend into the urothelium, but not on the urothelium itself. Secondly, several groups have reported problems with the specificity of TRPV1 antibodies and with non-specific immunoreactivity of urothelial tissue [6,18]. Thirdly, differences in cell culture techniques and (de)differentiation of cultured cells could influence the expression of TRPV1. Kullman et al. [9] reported considerable variability between different cover slips in the percentage of urothelial cells that responded to capsaicin, ranging from 0 to >60%, suggesting an influence of culture techniques on functional TRPV1 responses. Finally, species differences may also contribute to the apparent differences between these groups.

The functional role of TRPV1 in the bladder has been studied using $Trpv^{-/-}$ mice. In comparison to normal mice, these *Trpv1* knockouts had a higher frequency of non-voiding bladder contractions and had reduced reflex voiding under anaesthesia, but not in awake animals [19]. Moreover, $Trpv^{-/-}$ mice exhibit reduced firing of bladder afferents in response to bladder distension [20], indicating a role for TRPV1 in controlling the excitability of bladder sensory neurones.

TRPV1 is considered a promising target for functional disorders of the LUT because (i) activation of TRPV1 induces pain behaviour and detrusor overactivity (DO) in rodents [21], (ii) activation of TRPV1 in isolated bladder strips causes detrusor contraction via the release of neuropeptides and prostaglandins from afferent C-fibres [22], (iii) $Trpv1^{-/-}$ mice do not develop cystitis-induced DO and pain behaviour [23,24], (iv) TRPV1 expression is upregulated during pathological conditions, such as neurogenic DO (NDO) [25].

Several strategies have been developed to target TRPV1 (Fig. 1): (i) desensitisation of TRPV1 expressing afferents by TRPV1 agonists, (ii) directly inhibiting TRPV1 by TRPV1 antagonists and (iii) selectively inhibiting depolarisation of TRPV1-expressing afferents by TRPV1 permeable sodium (Na^+) blockers.

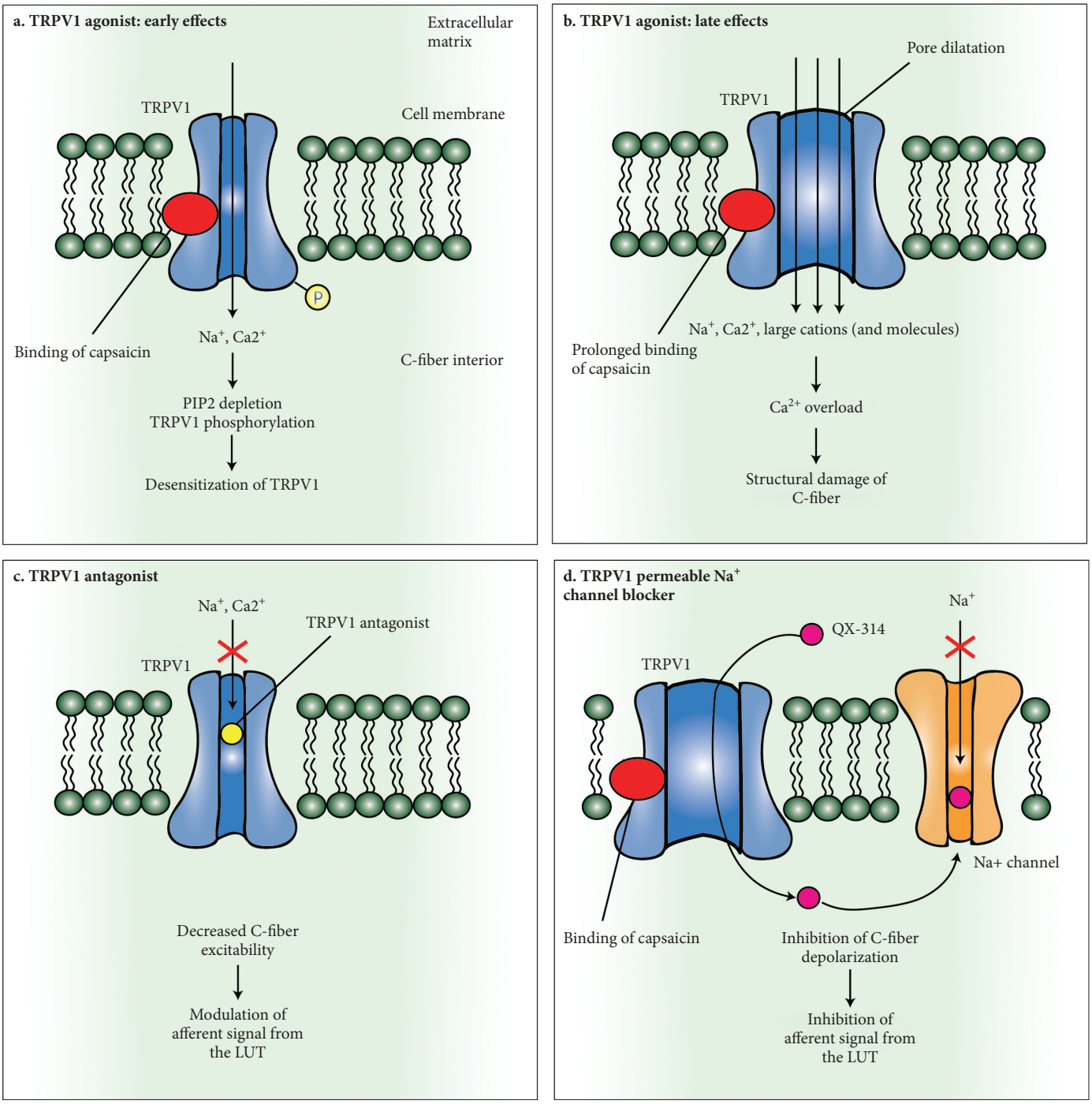
TRPV1 as a Pharmaceutical Target?

Agonists: pre-clinical data

Pharmacological experiments in cats and rats show that the TRPV1 expressing C-fibres are silent under physiological conditions but may become activated by bacterial or chemical irritants in the bladder giving rise to bladder hyperreflexia [26]. Moreover, these C-fibres become active and serve as the afferent arc for the spinal reflex that causes NDO in spinal cord-injured animals [27]. Interestingly, desensitisation of these C-fibres by systemic capsaicin treatment was able to inhibit cystitis-induced detrusor hyperreflexia in rats [26] and to successfully treat NDO in spinal cord-injured cats by interrupting the pathological reflex [28].

Activation of TRPV1 in C-fibres produces a biphasic response (Fig. 1). The immediate effect is stimulatory with transmission of sensory impulses from the periphery to the CNS (sensed as a painful irritation) and a peripheral release from the receptor terminals of neuropeptides including substance P and CGRP (leading to detrusor contraction and neurogenic inflammation). After exposure to high concentrations of capsaicin, afferent C-fibres show long lasting functional desensitisation and even morphological evidence of structural damage [27].

Fig. 1 Different mechanisms of TRPV1 modulation. (**a**) Short administration of TRPV1 induces desensitisation of the channel via not fully understood mechanisms including phosphorylation and phosphatidylinositol 4,5-bisphosphate (PIP2) depletion. This desensitisation causes a reduced excitability of the C-fibre. (**b**) Long-term administration of a TRPV1 agonist causes structural damage of C-fibres and even ablates them. Prolonged binding of capsaicin induces pore dilatation, which facilitates the entry of $Ca2^+$ and other large cations leading to cell death. (**c**) TRPV1 antagonists block opening of the channel making the C-fibres less responsive to TRPV1 activating stimuli. (**d**) QX-314 enters the C-fibre through the dilated pore of activated TRPV1 channels when co-administered with a TRPV1 agonist. From within the intracellular compartment QX-314 will block Na^+-channels hereby inhibiting depolarisation of the neurone and afferent signalling from the LUT.



Clinical development of TRPV1 agonists

Humans have been using capsaicin for thousands of years to 'spice up' their diet without long-term side-effects. However, systemic administration of capsaicin or its ultrapotent analogue RTX at higher doses induced several undesirable effects on blood pressure, breathing and other reflex pathways in preclinical models [29]. Therefore, most clinical trials with TRPV1 agonists have focussed on local delivery methods. Clinical development of several molecules to target pain relief, such as Adlea (ALGRX-4957, an injectable preparation of capsaicin) and Civamide [Zucacapsaicin, a cis-isomer of capsaicin that can be formulated for oral, nasal or topical (cream or patch) use] is already in an advanced stadium and Qutenza® (NGX 4010, capsaicin 8% patch), has recently been approved by the USA Food and Drug Administration (FDA) and European Medicines Agency (EMA) for treatment of post-herpetic neuralgia.

Since the initial description by Maggi et al. [30] about the use of intravesical capsaicin in humans, several randomised controlled trials (RCTs) have confirmed the alleviation of clinical and urodynamic symptoms after intravesical vanilloid therapy. In a double-blind placebo-controlled trial comprised of 20 patients with spinal cord injury with NDO, intravesical instillation of 30 mg capsaicin dissolved in 30% ethanol resulted in a significant improvement in clinical and urodynamic parameters, with a significant decrease in 24-h voiding frequency and maximum detrusor pressure and an increase in maximum cystometric capacity [31]. Similar results were obtained in another RCT, although at the expense of significant initial side-effects, including burning pelvic pain, sensory urgency, flushes, haematuria and autonomic hyperreflexia [32]. Using capsaicin dissolved in glucidic solvent instead of ethanol had similar short-term efficacy in patients with NDO with better tolerance after the instillation [33]. However, one small placebo-controlled crossover study did not show a significant improvement in NDO after intravesical capsaicin treatment [34].

In an attempt to improve efficacy and reduce side-effects, the ultra-potent TRPV1 agonist RTX has also been tested in several clinical trials. Two RCTs showed significant improvement in bladder capacity and incontinence grade in neurologically impaired patients with RTX compared with placebo [35,36]. Two RCTs comparing RTX vs capsaicin reported improved long-term efficacy [37], less adverse events and superior urodynamic and clinical improvement with RTX [38]. However, it should be remarked that, due to lack of stable formulations, RTX solutions differed amongst the aforementioned RCTs, which may have influenced the outcomes [39,40].

In conclusion, intravesical vanilloids appear to improve symptoms in ≈80% of patients with NDO but produce significant adverse effects [41,42]. The side-effect profile, the problems with stability of certain formulations and the success of other therapies such as intradetrusor injections of botulinum toxin have hampered the commercialisation and widespread use of intravesical therapy in clinical practise. Nevertheless, this therapy remains a valid option in therapy resistant NDO.

Intravesical vanilloids have also been tested as treatment for interstitial cystitis/painful bladder syndrome (IC/PBlS). Capsaicin did not significantly improve pain scores in patients with severe pelvic pain compared with placebo [43]. Similarly, a single administration of RTX did not significantly improve symptoms in a double-blinded RCT with 163 patients with IC [44]. In contrast, a recent pilot study showed that combined therapy of hydrodistention with intravesical RTX significantly relieved pain in patients with IC/PBlS [45].

Two RCTs have tested the effects of RTX in patients with idiopathic DO (IDO). One study showed a significant reduction of grade of incontinence after multiple RTX instillations [46], but the other showed no significant improvement in women with IDO and urgency incontinence [47]. In patients with overactive bladder with refractory urgency, instillation of a single dose RTX resulted in a significantly decreased number of frequency episodes and a subjective improvement in symptoms in 69% of patients [48]. Thus, despite good results in the treatment of NDO, the efficacy of TRPV1 agonists in the treatment of IC/PBlS and IDO remains debatable.

Antagonists: pre-clinical data

The effects of TRPV1-desensitising agonists and TRPV1 antagonists are not equivalent. Agonists do not merely desensitise TRPV1 channels, but also desensitise or even ablate TRPV1-expressing neurones (Fig. 1), whereas TRPV1 antagonists selectively inhibit TRPV1 activation. This implies that not only the therapeutic effect but also the side-effect profile is different between both classes of drugs.

In several animal models of LUT dysfunction, selective TRPV1 antagonists have proven to be promising candidates for drug therapy. GRC6211, an oral TRPV1 antagonist, reduced bladder hyperactivity in mice with lipopolysaccharide (a bacterial toxin)-induced cystitis [49] and in rats with NDO after spinal cord injury [50]. In rats, JTS653, another selective TRPV1 antagonist, attenuated acetic acid-induced bladder overactivity and blocks afferent neuronal transmission [51]. Similarly, systemic administration of JNJ17203212 increased the volume threshold for micturition in rats and inhibited both capsaicin and citric acid-induced DO [52].

Unfortunately, it became rapidly clear that the first generation of TRPV1 antagonists cause an increase in body temperature

(hyperthermia), both in animal models and in humans. Hyperthermia is an on-target effect, reflecting that TRPV1 is tonically active and plays a key role in body temperature regulation [29].

Clinical development of TRPV1 antagonists

In contrast to the relative lack of interest in TRPV1 agonists, pharmaceutical companies have very extensively pursued pharmacological blockade of TRPV1. However, despite the initial excitement only a few of these antagonists have advanced to clinical trials, since a Phase II study with AMG517 was discontinued because of marked and persistent hyperthermia (up to 39 °C) after exposure to single doses of this potent antagonist [53].

Similarly, XEND0501, an oral TRPV1 antagonist that was developed as a novel therapy for overactive bladder, was well tolerated in a Phase I clinical trial, but also provoked a dose-related increase in body temperature [54]. Unfortunately, due to early termination of most of these trials, the clinical effect of TRPV1 antagonists on pain or on bladder overactivity remains elusive.

Efforts to develop so called 'second generation' TRPV1 antagonists that do not block all modalities (activation by capsaicin, heat and low pH) of TRPV1, but selectively inhibit capsaicin activation have been reported not to increase body temperature [55]. The therapeutic effects of these new generation TRPV1 antagonists on pain perception or bladder function are still unknown. Alternatively, intravesical administration of TRPV1 antagonists might be a way to overcome systemic side-effects, although data about the efficacy of intravesical TRPV1 antagonists are currently lacking.

TRPV1 permeable Na^+ channel blockers

Recently, a novel strategy has successfully been used in rodents to selectively block TRPV1-expressing nociceptors. In this approach, TRPV1 is used as an 'entry gate' rather than the 'end-target'. QX-314, a positively charged derivative of lidocaine has no effect on neuronal Na^+ channels when applied extracellularly (as it cannot infiltrate lipid membranes) but does block Na^+ channels when applied intracellularly. Interestingly, when QX-314 is co-applied with capsaicin, it permeates inside the cell through activated TRPV1 channels and thus blocks Na^+ channels only in capsaicin-sensitive nociceptive afferents. This approach was successfully used to produce prolonged local anaesthesia to mechanical and thermal noxious stimuli in rats [56]. In efforts to avoid capsaicin-mediated nociceptive behaviour, anaesthetic agents with TRPV1-activating properties, such as isoflurane and lidocaine, have successfully been used to produce prolonged nociceptor-selective block when co-applied with QX-314 [57,58]. A recent study in rats showed that a triple cocktail of capsaicin, lidocaine and XQ-314 induced a near complete blockade of incision-induced hypersensitivity for several days, hereby confirming the clinical potential of this approach. However, this cocktail produced delayed mechanical hypersensitivity for several weeks due to neurotoxicity, underscoring the importance of appropriate safety screening of these new drug combinations, as safe doses of local anaesthetics may be toxic in combination with TRPV1 ion channel activators [59].

This approach appears very promising to attack LUT dysfunction with increased C-fibre activity, including NDO and PBlS, but must first be shown to be effective and safe in animal models and humans.

TRPV4

TRPV4 is a multimodal cellular sensor that can be activated by diverse physical and chemical stimuli, including moderate heat, cell swelling and binding of endo- and exogenous chemical ligands [60]. TRPV4 is expressed in numerous human tissues where it has a versatile role including mucociliary transport in cilia of the fallopian tubes and airways, bone remodelling in osteoclasts, osmoregulation in neurones of the circumventricular organs in the brain, and vascular function in endothelial cells [60]. Mutations in the TRPV4 gene are causative for several hereditary neuropathies and skeletal dysplasias, such as Charcot-Marie-Tooth disease type 2C, congenital distal spinal motor neuropathy and brachyolmia type 3 [60].

TRPV4 in the LUT

Functional TRPV4 expression has been shown in bladder urothelial cells in mice, rats, guinea-pigs and humans [9,12,61,62]. TRPV4 is located predominantly on the surface of the basal urothelial cell layers, in close proximity to the adherence junctions [60,61]. Lower levels of TRPV4 expression have also been detected in detrusor smooth muscle in mice and sensory nerve fibres [63].

In vitro experiments on cultured urothelial cells showed that cells from *Trpv4*$^{-/-}$ mice had attenuated Ca^{2+}-influx and ATP release in response to stretch compared with cells from control mice [14]. Moreover, isolated *Trpv4*$^{-/-}$ bladders showed reduced stretch-evoked ATP release [61].

In vivo, mice lacking the functional TRPV4 protein exhibit an abnormal voiding pattern and a lower frequency of voiding contractions compared with controls [61]. Moreover, intravesical infusion of GSK1016790A, a potent TRPV4 agonist is able to induce increased afferent firing of capsaicin-insensitive C-fibres and bladder hyperactivity in rodents [63,64], whereas, pharmacological blockade of TRPV4 by systemic administration of the TRPV4 antagonist HC067047 increases bladder capacity and reduces micturition

frequency in mice [65]. These findings support the hypothesis that TRPV4 acts as a mechano-sensor in urothelial cells that communicate with the underlying afferent nerves [60]. In a rat model of stress-induced bladder dysfunction, rats that were exposed to 7 days of repeated variate stress had a significantly smaller bladder capacity and an increased urinary frequency. In these rats, TRPV4 expression was significantly elevated at the mRNA and protein level in urothelium, but not in detrusor tissue [66].

Interestingly, patients with Charcot-Marie-Tooth disease type 2C caused by gain of function mutations in the TRPV4 gene have a high incidence of urinary urgency and incontinence [67]. It is unclear whether these overactive bladder symptoms are a consequence of increased TRPV4 activity or of the neurological disease.

TRPV4 as a Pharmaceutical Target?

Several pharmaceutical companies have patented TRPV4-targeted drugs for various diseases, but to date no clinical studies have been reported [68].

TRPV4 agonists

Intravesical instillation of the TRPV4 agonist GSK1016790A induced detrusor hyperactivity by selective activation of TRPV4 in mice [63]. Moreover, this drug is able to increase the contractility of isolated detrusor strips from rats with experimentally induced detrusor underactivity [69]. Unfortunately, systemic administration of GSK1016790A causes endothelial dysfunction with profound circulatory collapse and death in rodents [70]. Therefore further development of TRPV4 agonists has been abandoned.

TRPV4 antagonists

TRPV4 inhibitors may represent an interesting drug class for the treatment of LUT storage disorders. Systemic administration of HC067047, a selective antagonist of TRPV4, significantly reduced pollakisuria and increased the functional bladder capacity in rats and mice with cyclophosphamide-induced cystitis. HC067047 has similar effects in healthy animals, but the effects were significantly smaller, supporting a role for TRPV4 in the development of cystitis-induced bladder hyperactivity [65]. Moreover, intravesical administration of HC067047 significantly reduced the urinary frequency in rats with repeated variate stress-induced bladder dysfunction [66].

Interestingly, no significant effects of systemic treatment with HC067047 on various parameters, including core body temperature, heart rate, locomotion, motor coordination, fluid intake, or thermal selection behaviour were detected, suggesting these drugs have a good side-effect profile [65]. Similarly, systematic administration of GSK2193874, another selective orally available TRPV4 blocker, had no significant adverse effects in a rodent model for heart failure [71]. GlaxoSmithKline very recently published the protocol of the first Phase I clinical trial with a systemic TRPV4 antagonist (GSK2798745) to test its safety and tolerability in healthy subjects and patients with stable heart failure. Results of this study are awaited eagerly [72].

TRPM8

TRPM8 was identified as a marker of prostate cancer, before it was described as a sensory ion channel belonging to the TRP superfamily. TRPM8 activity is increased by cool temperatures (8–25 °C) and by chemicals that provoke 'cool' sensations, such as menthol and icilin. G proteins, lipid messengers and polyunsaturated fatty acids also modulate TRPM8 activity [73]. As a cold sensor in the body, TRPM8 is predominantly expressed in a small subpopulation of dorsal root ganglion (DRG) and trigeminal neurones that do not express TRPV1 [73].

TRPM8 in the LUT

TRPM8 expression was shown in a subset of small fibres from S2–S4 DRG neurones that innervate the LUT in human [74] and rat [75]. Despite earlier reports of urothelial TRPM8 expression in rats [9] and humans [76], TRPM8 expression in urothelium remains highly controversial. Several recent studies were unable to detect TRPM8 mRNA, protein or functional responses in mouse [6,12,14] and human [16] urothelium.

Intravesical infusion of the TRPM8 agonist menthol facilitates the micturition reflex in guinea-pigs and rats [77,78] and sensitised the bladder to infusion of cold (4 °C) saline [77]. Conversely, pharmacological blockade of TRPM8 decreased the frequency of volume-induced bladder contractions in rats [79].

As a cold sensor, TRPM8 has been suggested to play a role in the ice-water test [77,80]. In the pre-MRI era, this test was used to determine the level of neural injury in patients with NDO. Instillation of ice-cold water evoked bladder contractions in patients with upper but not with lower motor neurone lesions [81]. Importantly, all these publications used menthol as a TRPM8 specific agonist. Nevertheless, it has been clearly shown that menthol is not a specific agonist for TRPM8 but also activates the noxious cold sensor TRPA1 and other cellular targets [82,83].

It is well known that cold weather can precipitate urinary urgency symptoms in patients [84]. Similarly, environmental cold induces DO in rats and this effect is counteracted by systemic administration of BCTC, a TRPM8 selective channel antagonist [85].

TRPM8 as a Pharmaceutical Target?

TRPM8 agonists

TRPM8 agonists are considered important therapeutic compounds for attenuating pain and inducing apoptosis of cancer cells expressing TRPM8. Menthol-containing capsules are currently being tested in a Phase III trial as treatment for hypertension, whereas a menthol-containing cream will go into Phase III testing for pain relief of osteoarthritis [68]. D3263, an orally bioavailable TRPM8 agonist developed by Dendreon, inhibits the growth of tumour cells expressing TRPM8 *in vitro*. This compound has already completed Phase I testing and the company recently announced the initiation of clinical studies on patients with advanced solid tumours [68].

Based on the observation that TRPM8 activation facilitates the micturition reflex [77,78], TRPM8 agonists may be beneficial in patients with detrusor underactivity. However, currently there is no strong evidence to support this strategy.

TRPM8 antagonists

Several pharmaceutical companies have made tremendous efforts in the development of TRPM8 antagonists as analgesics, antitumor agents and for the treatment of overactive bladder [68]. In preclinical studies, TRPM8 antagonists reduce cold stress-induced DO in rats and decrease voiding frequency in rats with cyclophosphamide-induced cystitis [85,86]. In contrast to TRPV1 antagonists, pharmacological blockade of TRPM8 causes hypothermia in rodents [87]. To date, none of these TRPM8 antagonists has made it to clinical trials.

TRPA1

TRPA1 functions as a broadly tuned sensor for noxious stimuli in a subpopulation of TRPV1-expressing primary afferent nociceptive neurones. The channel can be activated by a myriad of stimuli, such as intracellular Ca^{2+} and Zn^{2+}, noxious cold temperature (<17 °C) and a wide variety of chemical irritants [88].

TRPA1 in the LUT

Recently, there has been great interest in the functional role of TRPA1 in the urinary bladder, the intestine and the colon. As a mediator of inflammation and pain, TRPA1 is considered a potential target in the treatment of visceral hypersensitivity syndromes, e.g. inflammatory bowel disease, PBlS and overactive bladder. In the bladder, TRPA1 channels are expressed in sensory nerve endings from lumbosacral DRG neurones, but not in the urothelial cells [6,12,14,88]. TRPA1-expressing C-fibres constitute ≈50% of all bladder-innervating sensory neurones and mostly express CGRP, substance P and TRPV1.

TRPA1 functions as a sensor of noxious stimuli in the bladder. Intravesical instillation of TRPA1 agonists, e.g. allyl isothiocynate or cinnamaldehyde, stimulates bladder emptying by inducing urinary frequency *in vivo* [89,90]. This is supported by *in vitro* experiments showing that TRPA1 agonists cause a graded contraction of rat urinary bladder strips [91] but induce relaxation of phenylephrine pre-contracted urethral muscle strips [92]. Interestingly, TRPA1 can be activated by acrolein, the metabolite of the chemotherapeutic drug cyclophosphamide that is responsible for inducing haemorrhagic cystitis. Thus, TRPA1 may, at least in part, be responsible for the development of haemorrhagic cystitis in patients with cancer treated with cyclophosphamide. Bacterial toxins and metabolites, e.g. lipopolysaccharides and H_2S, have recently been shown to activate TRPA1, suggesting that TRPA1 also plays a role in the early detection and elimination of urinary pathogens [93]. In addition, we recently reported that TRPA1, rather than TRPM8 might be the actual cold sensor in the bladder cooling reflex [94].

TRPA1 as a Pharmaceutical Target?

TRPA1 antagonists

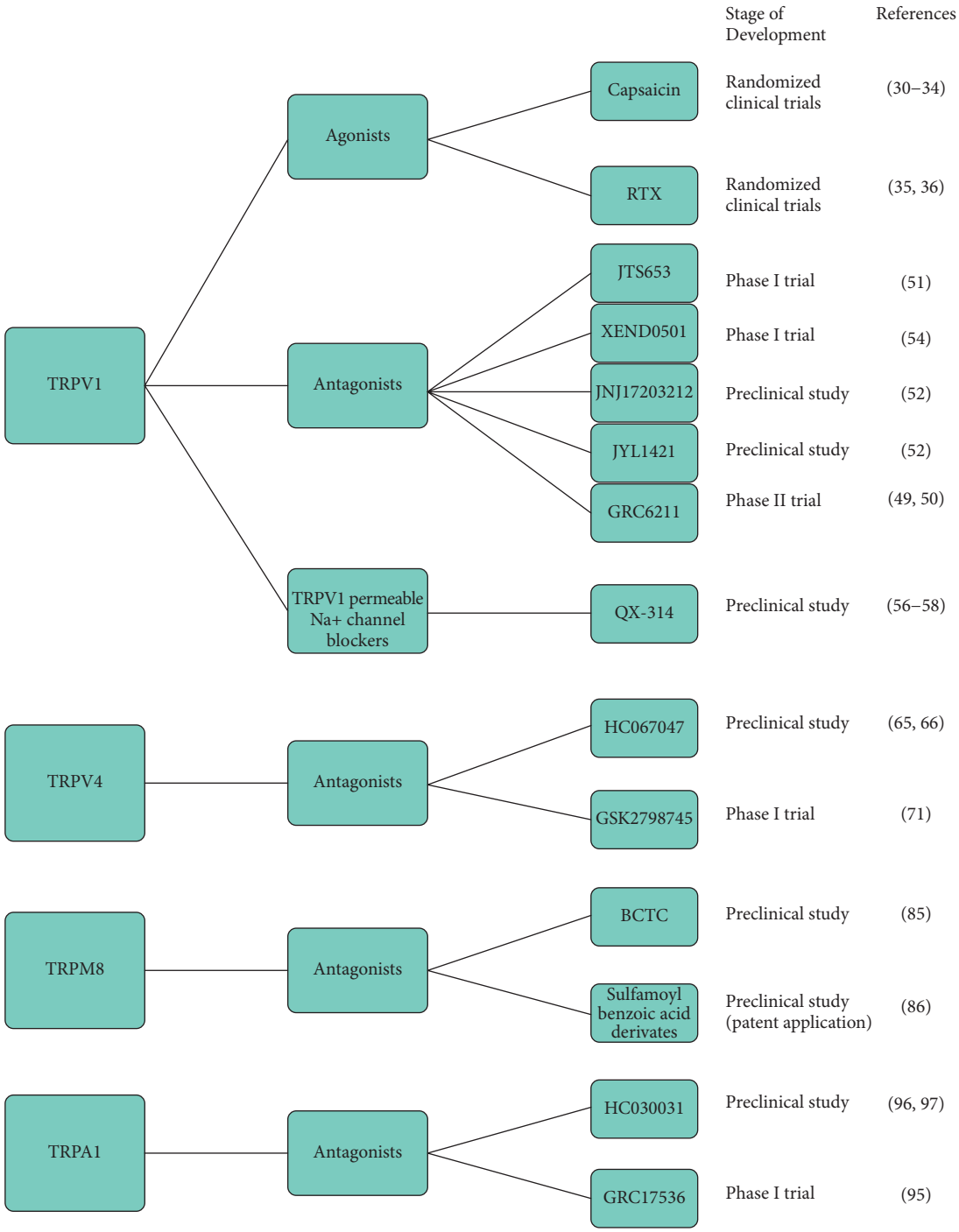
Pharmaceutical companies have patented multiple TRPA1 antagonists. Although these drugs show efficacy in various animal models for pain and hyperalgesia, they await clinical validation [68]. One compound, GRC17536, has successfully completed a Phase I clinical trial and a Phase II clinical trial in patients with refractory chronic cough is currently ongoing [95].

Another TRPA1 antagonist, HC030031, has been proven effective to ameliorate cystometric parameters in rats with NDO after spinal cord injury. In these rats, TRPA1 expression in DRG ganglia was upregulated at the protein and mRNA level and the effects of the TRPA1 antagonists could be mimicked by TRPA1 RNA knockdown [96]. In a visceral pain model, using cyclophosphamide-induced cystitis, HC030031 effectively alleviated cystitis induced bladder hyperalgesia [97]. The plethora of TRPA1 antagonists still awaits clinical validation.

Other TRP Channels in the LUT

In addition to the TRP channels discussed above, many other TRP channels are expressed throughout the bladder wall. In the urothelium of mice, mRNA expression of 22 different TRP channels could be detected, albeit at variable levels [6]. TRPV2, a close relative of TRPV1, is expressed in murine, rat and human urothelial cells [12,19,98]; however, the exact physiological role remains poorly understood due to lack of specific pharmacology and antibodies. TRPM4 is expressed at

Fig. 2 Examples of TRP channel modulating compounds with potential to treat DO and their stage of development.



the apical membrane of umbrella cells and in rat detrusor cells where they may regulate smooth muscle excitability and contractility [99]. Also functional TRPM7 expression has been demonstrated in mouse and human urothelial cells [12,16], although the functional role of this ubiquitously expressed ion channel remains elusive.

Conclusions

At the beginning of the new millennium the scientific and medical world was 'high' on TRP channels, as this new ion channel family emerged as an exciting new therapeutic target. Since then, a lot of scientific, industrial and clinical effort has

been put into the characterisation and pharmaceutical targeting of TRP channels.

Although it is clear that several TRP channels play key roles in normal and pathological LUT physiology, many questions and controversies remain about their exact localisation, structure and function. The main reasons for these apparent difficulties are a lack of antibody and (ant)agonist specificity, methodological variability and interspecies differences, inherent to the relative youth of this research field.

In recent years, a large constellation of compounds has been developed that target TRP channels and, although therapeutic indications are numerous, only a few of them have advanced into clinical trials. TRPV1 is by far the best pupil in the class with the recent FDA approval of the TRPV1 agonist, capsaicin, to treat post-herpetic neuralgia. For the treatment of LUT diseases the clinical breakthrough of TRP channel modulation is still awaited, notwithstanding that TRPV1 agonists have shown good efficacy in patients with NDO. The first generation of TRPV1 antagonists suffered from their side-effect profile, inducing hyperthermia, but a new generation of antagonists is being developed to tackle these problems. These insights can assist further development of other TRP (ant)agonists. Alternatively, intravesically applied TRP-targeting drugs may be used to minimise these systemic adverse events. Compounds modulating other TRP channels in the LUT (TRPV4, TRPM8 and TRPA1) are currently being tested in pre-clinical studies and Phase I clinical trials (Fig. 2) [30–36,49–52,54,56–58,65,66,71,85,86,95–97], so hopefully these TRP channel modulators can realise the expectations of becoming **t**ruly **r**evolutionary **p**harmacotherapies.

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Conflict of Interest

D.D.R. is consultant and/or investigator for Astellas, Medtronic, Xention, and Allergan.

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Abbreviations: CGRP, calcitonin gene-related peptide; (I)(N)DO, (idiopathic) (neurogenic) detrusor overactivity; DRG, dorsal root ganglion; FDA, USA Food and Drug Administration; IC, interstitial cystitis; LUT, lower urinary tract; PBlS, /painful bladder syndrome; RCT, randomised controlled trial; RTX, resiniferatoxin; TRP(A)(M)(V), transient receptor potential (ankyrin) (melastatin) (vanilloid).

